ST. CLAIR RIVER ORGANICS STUDY:

FISH TOXICITY AND TAINTING EVALUATIONS FOR SELECTED INDUSTRIAL EFFLUENTS

1977

Ministry of the Environment
Ontario

The Honourable
Andrew S. Brandt
Minister

Gérard J. M. Raymond
Deputy Minister

ST. CLAIR RIVER ORGANICS STUDY:

FISH TOXICITY AND TAINTING EVALUATIONS

FOR SELECTED INDUSTRIAL EFFLUENTS

1977

Limnology and Toxicity Section
Water Resources Branch
Ministry of the Environment
Box 213, Rexdale, Ontario

LTS 81-1

ALQE

ST. CLAIR RIVER STUDY - EXPLANATORY NOTE

FISH TOXICITY AND TAINTING EVALUATIONS FOR SELECTED EFFLUENTS

The three-fold purpose of the study was to examine effluent from five Sarnia area petro/chemical industries and determine the following effects on fish:

1. mortality to fish
2. capacity to taint fish
3. to analyze the fish and effluents for chemical components that may cause the mortality and tainting.

Industries from the Sarnia area selected for sampling as a result of preliminary investigation included Esso Chemical Company, Imperial Oil Limited, Dow Chemical Company, Ethyl Corporation LImited and Allied Chemical Company Limited.

Rainbow trout were exposed to varying levels and concentrations of effluent samples collected from the five companies. Trout were placed in containers of effluent-solution for 96-hours. Fish mortality was then tabulated in this 96-hour static bioassay.

Another fish test performed was the 48-hour flow-through tainting bioassay. Here various percentages of industrial effluent were added every two minutes to individual aquaria holding rainbow trout.

The 96-hour static bioassay demonstrated that one effluent from Esso Chemical was lethal. Fifty percent of the fish survived at a 60% concentration. The 48-hour flow-through test on Esso Chemical effluent demonstrated similar results. With Allied Chemical effluent, 90% of the fish survived 100% concentrations in the 96-hour static test. The three remaining effluents were non-lethal, but produced signs of stress on the test fish.

All effluents studied had the capacity to taint fish. Two of the three effluents were moderate in their tainting capacity and the remaining three were severe.

Commonly used water pollution tests like biological oxygen demand (BOD) and phenolic testing indicated that effluent samples were relatively clean. BOD robs water of life-giving dissolved oxygen, while phenols are contaminants associated with the petro/chemical industries which can taint water and fish.

Sophisticated organic testing was completed, using the Ministry's gas chromatograph/mass spectrometer (GS/MC) which is actually two tools brought together to function as one in determining and measuring organic compounds. The GS/MC determined that fish can accumulate organic contaminants to a much higher concentration than those present in the effluents of the five industries. Organic compounds may be measured in the parts per billion range in the effluent but may be accumulating in the fish at the much higher rate of parts per million, representing a thousand-fold increase.

The only conclusion drawn from the study was that the effluent from all industries sampled can cause taste and odour problems in fish. Clearly the fish used in the experiments because of the tainting problem were unfit to eat but the public health implications of consuming fish from the river contaminated with industrial organic compounds would require further study. Effects on the reproduction, growth, basic physiology and biochemistry of fish were undetermined.

ORGANIC COMPOUNDS FOUND IN FISH

ST CLAIR RIVER

Benzene
Toluene
Xylenes
Trimethylbenzene
Diethylbenzene
Acetone
Aliphatics
Styrene
Chloroform
Carbon Tetrachloride
Cumene
Napthalene

LAKE HURON

Benzene
Toluene
Aliphatic hydrocarbons

LAKE ST. CLAIR

Chloroform
Tetrachloroethylene
Benzene
Toluene
Xylene
Diethylbenzene
Butanone
Acetone
Dimethyl disulphide
C_5-aldehyde
Aliphatic hydrocarbons

TABLE OF CONTENTS

FOREWORD

An in-depth investigation of organic chemicals in industrial effluents along the St. Clair River commenced in 1973. The adoption of gas chromatography/mass spectrometry analytical methodologies, by the Laboratory Services Branch of the Ministry of the Environment, made the detection of low concentrations of such compounds possible. In a preliminary report, "Organic Compounds of Industrial Origin in the St. Clair River", developed as an internal working document in January of 1977, positive identification was made of some 47 organic compounds discharged to the St. Clair River.

The St. Clair River Organics Study Group was formed early in 1977 to further investigate and confirm the presence and significance of the organic compounds identified, particularly in relation to possible effects on public water supplies downstream and the potential bio-accumulation of such compounds by fish resident in or frequenting this important waterway. Also, it was decided that a comprehensive assessment of water quality conditions and aquatic life was warranted to permit a comparison with unpublished biological data collected in 1968.

Representation on the Study Group included staff from various branches of the Ministry of the Environment and the Special Studies and Services Branch of the Ministry of Labour. Early in its deliberations, the Study Group identified the following goal:

To assess the presence and significance of organic chemical compounds in the St. Clair River system and to establish from this programme recommendations for control measures and further studies that may be required in relation to human health and environmental effects.

In order to accomplish the above goal, the following ten objectives were identified:

1. Identification and quantitation of organic compounds in industrial discharges, St. Clair River water, potable water supplies, bottom sediments and fish.

2. Completion of a literature search on the characteristics and effects of the organic compounds identified. Lethal, sublethal and synergistic effects of these compounds on human and aquatic life to be considered.

3. Determination of the significance of chlorinating trace amounts of these organic materials in industrial cooling water discharges, sewage plant effluents and in potable water supplies.

4. Determination of the types and concentrations of compounds causing fish toxicity and fish tainting problems.

5. Estimation of the dissolved organics concentration at any point in the river based on effluent quality and flow measurements.

6. Completion of benthic macroinvertebrate, aquatic plant, sediment and water quality studies on the river in terms of biological and common chemical indicators. Results to be compared to survey work carried out in 1968-69. Assessments of mercury levels in sediments to be completed.

7. Determination of the mutagenicity of organic compounds through quick-screening microbiological tests.

8. Establishment of whether biodegradation or alteration of organic compounds occurs through bacterial action in sediments.

9. Development of recommendations clarifying levels to which contaminants must be reduced, as a basis for necessary treatment and effluent controls.

10. Clarification of the need for additional research to establish potential dangers to human health or the natural environment where this information is lacking.

Work on the component studies related to these objectives progressed throughout 1977 and 1978 and into 1979. As a result of these efforts the following reports were published to form an integrated series covering the objectives defined previously.

1) Identification and Quantitation of Organic Compounds.
2) Fish Toxicity and Tainting Evaluations for Selected Industrial Effluents.
3) Waste Dispersion
4) Biological Surveys 1968 and 1977.
5) The Detection of Mutagenic Activity; Screening of Twenty-three Compounds of Industrial Origin.
6) Biodegradation of Organic Compounds.

Wherever possible, each of these reports will present conclusions and advance recommendations to achieve an improvement of water quality in the St. Clair River system or to clarify additional research requirements in regard to potential human health or environmental effects.

It is anticipated that subsequent reports dealing with the potential carcinogenicity of industrial organic compounds in relation to the St. Clair River will be issued by the Ministry, since these particular components will be the subject of further study by the Microbiology Section of the Laboratory Services Branch.

SUMMARY

Five industrial effluents discharging into the St. Clair River were evaluated for fish lethality and tainting.

Only the Esso Chemical Pressure Sewer proved to be lethal to rainbow trout while the Imperial Oil Bio-oxidation Plant Effluent, Dow Chemical Third Street Sewer, Ethyl Corporation Final Effluent and Allied Chemical Final Effluent were essentially non-lethal.

All effluents produced fish tainting, with discharges from Esso Chemical, Imperial Oil and Dow Chemical being the most potent (<5% v/v). Those from Ethyl Corporation and Allied Chemical were less potent (26% v/v).

Compounds associated with lethality, tainting and/or bioconcentration included napthalene, methylnapthalene, toluene, styrene, benzene, xylene/ ethylbenzene, diethylbenzene and dichlorobenzene.

INTRODUCTION

The St. Clair River flows in a southerly direction from Lake Huron to Lake St. Clair, the Detroit River and Lake Erie. The river's dredged shipping channel carries approximately 90% of 180,000 C.F.S. flow. The two shallower and slower shoreline streams carry the balance of the flow.

The very intensive development of petroleum and petrochemical based industries along the Canadian Shore discharge into this relatively small slow-moving shoreline stream. These industrial discharges have led to some recognized problems in the receiving water such as floating scums and particles, oil slicks, tainting of fish as well as foul, oily, chemically smelling water (1). Information released by the Lambton Industrial Society (2) showed that the nearshore water of the St. Clair River had the capacity to impair fish flavour and fish growth. These studies provided the background and rationale for the present study which assesses the short term lethality and fish tainting capacity of five industrial effluents.

Consultation with regional staff plus subjective odour ranking of the effluents led to the selection of discharges for study. Other factors that were considered were that the effluents selected should represent a broad spectrum of the various industrial processes without undue emphasis on one industrial type. Toxic effluents which were already under intensive investigation in other studies were excluded. Additional criteria considered were that the effluents could represent a major source of organics to the river. The five effluents selected are identified below and are described by Bonner and Meresz (10).

1) Esso Chemical Co., Ltd., - Pressure Sewer
2) Imperial Oil Ltd., - Bio-oxidation Plant Effluent
3) Dow Chemical of Canada Ltd., Sarnia Works - Third Street Sewer
4) Ethyl Corporation - Final Effluent
5) Allied Chemical - Final Effluent

The techniques used in the examination of these effluents included 96-hour static bioassays, 48-hour flow-through bioassays, flavour assessment of experimental fish and gas chromatographic/mass spectrometric (GC/MS) examination of some of the fish and effluents.

METHODS

The basic procedure for these studies involved taking samples of the effluents and transporting these to Toronto where they were tested for fish lethality and tainting using rainbow trout (Salmo gairdneri). Effluent samples for bioassay and chemical analysis were collected concurrently.

Effluent Collections

All effluents used in this study were taken on a grab sample basis. The effluent samples that were to be used only for static, 96-hour lethality testing were put into polyethylene-lined 5 gallon plastic pails. All sampling lines, pumps and containers were thoroughly flushed before the container was filled. Once filled, all excess air was squeezed from the liner bag and the top tightly closed.

For the tainting exposure, 800 gallons of each effluent was collected. Five hundred gallons were pumped into a rinsed stainless steel tank and filled to minimize any head space. The remaining three hundred gallons were put into polyethylene-lined, 45 gallon drums. The drums were filled and tightly closed to minimize any head space. At the same time, samples were also taken and appropriately preserved for chemical analyses according to Ministry of the Environment procedures.

Table 1 summarizes the sample collection and test dates.

Dilution Water

The dilution water used in all bioassays was dechlorinated Toronto tap water. Dechlorination was achieved through the use of activated charcoal and ultra violet irradiation. During the period of the bioassays the dilution water had the following properties: hardness, 130 mg/l; alkalinity, 89 mg/l; chloride, 31.5 mg/l; sulphate, 32 mg/l; calcium 39 mg/l; magnesium, 8 mg/l; sodium, 12 mg/l; potassium 1.4 mg/l; total Kjeldahl nitrogen, 0.21 mg/l; free ammonia, 0.006 mg/l; nitrite 0.001 mg/l; nitrate, 0.459 mg/l; conductivity, 345 μmho/cm^2; total residual chlorine, 9 μg/l; pH 7.9.

Fish Stocks

Trout used in the static and flow-through bioassays were held for a minimum of 21 days in dilution water. The holding temperature for the fish

Table 1: Dates of Sample Collection and Testing

Site	Sample Collected	Static Bioassay	Flow-Through Bioassay	Taste Test
Esso Chemical				
Pressure Sewer	May 10/77	May 11/77	May 12/77	May 17/77
Imperial Oil				
Bio-oxidation Plant	July 12/77	July 20/77	July 20/77	July 26/77
Dow Chemical				
Third Street Sewer	May 31/77	June 1/77	June 1/77	June 7/77
Ethyl Corp.				
Final Effluent	Aug. 22/78	Aug. 23/78	Aug. 24/78	Sept. 7/78
Allied Chemical				
Final Effluent	June 21/77	June 23/77	June 22/77	July 5/77

stocks was 14-16^{o}C. Mortality during the holding period was less than 1%. The fish used in the static bioassays averaged 0.89 gms. while those in the flow-through test averaged 254 gms.

Toxicity Tests - Static Lethal

The samples were stored overnight at 15^{o}C. On the morning of the test the temperature, dissolved oxygen, pH and specific conductance was recorded. Ten fish were exposed per effluent concentration (50%, 70%, 100%) made up to 40 litres, aerated and held at 14-16^{o}C for 96-hours. Fish mortality checks were completed at 0.25, 0.5, 1, 2, 4, 8, 16, 24, 48 and 96-hours. Surviving fish were sacrificed and controls were weighed and fork length measured. The minimum loading rate of 0.5 litres per gram fish per day was exceeded in all tests (Table 2). Mortality and concentration data were plotted to estimate LC50 values by probit analysis.

Tainting Evaluation - Flow-Through Test

The effluent samples used in the tainting exposure were stored in two separate portions at 4^{o}C. One portion was stored in two 250 gallon refrigerated stainless steel tanks; the balance of the sample was stored in tightly closed 45 gallon drums in a 4^{o}C cold room. This latter portion was used to replenish effluent in the storage tanks.

Effluent flowed to a stainless steel basin where the temperature was adjusted to 15^{o}C. A modified Mount/Brungs diluter was used to deliver six concentrations (100% v/v, 50% v/v, 10% v/v, 5% v/v, 2 controls) to six 225-litre glass aquaria at 0.5 l/min. Temperature, dissolved oxygen, pH and conductivity in the exposure tanks were recorded at 0, 24 and 48-hours. Chloride concentrations in the exposure tanks were also monitored to confirm the diluter proportioning.

After the fish were added, a perspex lid was put on the tanks and the whole exposure apparatus draped in black plastic to minimize disturbances to the fish.

Fish were sacrificed after 48-hours, weighed, wrapped individually in aluminum foil and frozen in plastic bags. Fish from a common exposure were stored together for the tainting evaluation.

Table 2: Bioassay Loading: Static and Flow-Through Tests.

Collection Site	Static *(l/g/day)**	Flow-Through *(l/g/day)**	Total Flow *(l)*
Esso Chemical			
Pressure Sewer	0.95	0.70	1500
Imperial Oil			
Bio-oxidation Plant	1.12	0.56 *(slow diluter)*	870
Dow Chemical			
Third Street Sewer	0.61	0.71	1587
Ethyl Corp.			
Final Effluent	1.45	0.51 *(big fish)*	1280
Allied Chemical			
Final Effluent	0.70	1.10	1560

**Current practice requires at least 0.5 l/g/day (9).*

Tainting Evaluation - Taste Panel

Sufficient frozen fish exposed to the concentration series of a single effluent were prepared for the taste panel daily. This panel consisted of ministry staff who professed no dislike of fish and who could attend tasting sessions regularly.

Eight samples of fillet, each approximately 16 cubic cms. were taken between the dorsal and lateral line of each fish. Skin and large bones were removed. The samples were placed in new aluminum foil baking cups, covered tightly with aluminum foil, crimped to minimize loss of vapours and assigned numbers in a coded sequence. Thawed samples were baked for 15 minutes at 300°F, then taken to the test room where they were kept warm under a heat lamp for a maximum of twenty minutes prior to serving. Fish exposed to the Ethyl Corporation effluent were cooked in plastic petri dishes in a microwave oven.

The tasting sessions were held in an unoccupied air-conditioned room, scheduled one hour after meals. Panelists were asked to refrain from smoking prior to tests. Women panelists were asked to remove lipstick and all panelists washed their hands with odour-free soap before each taste session. All glassware and implements were washed with odour-free soap and rinsed with distilled water.

Each panelist was provided with a dish of unsalted crackers, waste container, paper cup, flask of rinse water and instruction sheet. Rinse water was made by adding one tablespoon of lemon juice to one litre of distilled water.

Panelists were presented with two samples at a time, identified only by number. They were instructed to smell the steam from each sample and note for future reference. The fish sample was tasted then discarded into the waste beaker. The numbered samples were tasted in turn with a cracker chewed and the mouth cleared with rinse water. Among the samples was one identified and one hidden control. The flavour of each sample was rated as one of the following.

0 - Flavour equal to or better than known control
1 - Slightly different from control
2 - Moderately different from control
3 - Strongly different from control
4 - Extremely different from control - repulsive

Along with this ranking of the flavour intensity, the panel was urged to provide any descriptive phrases that characterized the flavour.

The numerical flavour ratings for each treatment level were summed for comparison between controls and each treatment level. Significance from controls was determined using Wilcoxon's Modified Non-Parametric ANOVA, (3, 4). Tainting threshold concentrations were estimated by graphical interpolation.

Subsamples of frozen flesh were submitted for GC/MS analysis. The flesh was homogenized, suspended in water and purged with nitrogen. Effluent samples were also nitrogen purged for GC/MS analysis.

RESULTS

Toxicity

During the period of March 1976 to September 1978 bioassays were performed on the five selected discharges (Table 3). Imperial Oil Bio-Oxidation Plant Effluent, Dow Chemical Third Street Sewer and Ethyl Corp. Final Effluent samples were consistently non-lethal. Allied Chemical Final Effluent on a single occasion had an LC50 of 47% v/v while either no or minimal mortality was observed on five other occasions. The Esso Chemical Pressure Sewer was tested ten times during this period. On four occasions the discharge was non-lethal. The remaining six samples had LC50's ranging from 51% to 100%.

The five effluents collected for tainting evaluation were consistent with past toxicity results. The Esso Chemical Pressure Sewer had a 48-hour LC50 (flow-through) of 70% v/v while the other four discharges were non-lethal at 48-hours (Table 4).

Although most effluents were non-lethal, stress was observed in the fish through darkening of skin, loss of equilibrium, and surface breathing in the higher concentrations.

Tainting

All five effluents tested impaired fish flavour (Table 4).

Table 3: Summary of bioassay results for five discharges investigated from March 1976 to September 1978.

Date of Sample	Test Mode	96-hour LC50
Esso Chemical: Pressure Sewer Effluent		
13/04/76	U.A.	51%
28/06/76	U.A.	75%
19/07/76	U.A.	93%
25/10/76	A	non-lethal
18/04/77	A	non-lethal
10/05/77	A T.T.	60%
31/05/77	A	70%
21/06/77	A	non-lethal
12/07/77	A	non-lethal
11/07/77	A	100%
Imperial Oil Enterprises Ltd.,: Bio-Oxidation Plant Effluent		
28/06/76	U.A.	non-lethal
25/10/76	A	"
18/04/77	A	"
10/05/77	A	"
31/05/77	A	"
21/06/77	A	"
12/07/77	A T.T.	"
11/07/76	A	"
Dow Chemical of Canada Ltd: Third Street Sewer		
03/03/76	U.A.	non-lethal
21/06/76	U.A.	"
10/05/77	A	"
21/05/77	A T.T.	"
21/06/77	A	"
12/07/77	A	"
11/07/77	A	"
12/09/78	A	"
Ethyl Corporation: Final Effluent		
12/07/76	U.A.	non-lethal
10/05/77	A	"
31/05/77	A	"
21/06/77	A	"
12/07/77	A	"
11/07/77	A	"
22/07/78	A T.T.	"
12/09/78	A	"
Allied Chemical: Final Effluent		
19/07/76	U.A.	47%
10/05/77	A	non-lethal
31/05/77	A	"
21/06/77	A T.T.	10% mortality at 100%
12/07/77	A	non-lethal
12/09/78	A	"

U.A. = Unaerated bioassay - static test
A = Aerated Bioassay - static test
T.T. = Effluent subsample also used for taste testing - flow-through test.

Table 4: Lethality and tainting threshold concentration (v/v) observed in 48-hour flow-through bioassay.

Effluent	Duration of Exposure (hours)			Taint Threshold
	4-hours	24-hours	48-hours	
Esso Chemical				
Pressure Sewer	Non-lethal @ 100%	LC50 ≈87% v/v*	LC50 ≈70%*	<5%
Imperial Oil				
Bio-Oxid. Plant	Non-lethal @ 100%	Non-lethal @ 100%	Non-lethal @ 100%	<5%
Dow Chemical				
Third St. Sewer	"	"	"	<5%
Ethyl Corp.				
Final Effluent	"	"	"	26%
Allied Chemical				
Final Effluent	"	"	"	26%

*LC50 values are estimates, based upon four fish per concentration

Esso Chemical Pressure Sewer, Imperial Oil Bio-Oxidation Plant and Dow Chemical Third Street Sewer produced a significant fish taint ($\rho < 0.05$) at the lowest (5%) concentration tested. Ethyl Corporation and Allied Chemical Final Effluents produced a significant taint ($\rho < 0.05$) at the 50% concentration and the tainting threshold was estimated at 26% v/v.

Chemical

The routine effluent quality parameters measured on the five samples tested are summarized in Table 5.

For Esso Chemical Pressure Sewer, Imperial Oil Bio-Oxidation Effluent, Dow Chemical Third Street Sewer and Allied Chemical Final Effluent, the volatile organic components were measured by GC/MS and are summarized in Tables 6, 7, 8, 10.

Subsamples of fish flesh used in the tainting evaluation were also tested by GC/MS for volatile organics. Samples of raw flesh from fish exposed to 100% effluent from Esso Chemical Pressure and Imperial Oil Bio-Oxidation Effluent were tested. A series of flesh samples, both raw and cooked, were analyzed for volatiles in fish exposed to the full concentrations in the other three effluents. These results are tabulated in Tables 8, 9, 10.

DISCUSSION

Toxicity

The effluent samples collected for this study were representative of historical samples collected in that they were essentially non-lethal.

Only the Esso Chemical Pressure Sewer exhibited regular lethality while Allied Chemical was considered non-lethal despite one recorded LC50. Review of the conventional water quality parameters does not account for the observed lethality. The most likely toxic agents in the Esso effluent are napthalene (6 mg/l) and methylnapthalene (270 mg/l) based on a reported 24-hour LC50 for napthalene of 220 mg/l with mosquito fish (5). Since 1977 incorporation of charcoal treatment has reduced acute lethlity and organics in the Esso effluent.

Tainting

The fish tainting evaluation represents a sensitive tool to identify

Table 5: Effluent Quality Parameters for Samples used in the Taste Test

Effluent Source	Cl^- mg/l	BOD mg/l	COD mg/l	TOC mg/l	Phenol μg/l	pH	NH_3^-N mg/l	Conductivity μmohs/cm^2
Esso Chemical								
Pressure Sewer	68	19	88	16	75	8.8	<0.1	470
Imperial Oil								
Bio Oxid. Plant	176	5	59	19	20	7.6	7.7	900
Dow Chemical								
Third St. Sewer	60	28	41	6	40	9.1	<0.1	410
Ethyl Corp.								
Final Effluent	540	-	20	5	7	8.2	<0.1	1670
Allied Chemical								
Final Effluent	44	4	21	5	6	7.7	0.6	275

Table 6: Esso Chemical Pressure Sewer. Organic compounds identified in the effluent and fish exposed to the undiluted effluent for 48-hours.

	Effluent µg/l	Raw Fish* µg/kg
Benzene	4	88
Toluene	2	113
Xylenes	28	375
Styrene	8	310
Cumene + Ethyl toluene	4	175
Trimethyl benzenes	8	300
Naphthalene	6,000	250,000
Methyl naphthalene	270,000	1,150,000
Diethyl Benzene	10	1,200

* composite of fillets from 4 fish (fish died during exposure).

Table 7: Imperial Oil Bio-Oxidation Plant Organic compounds identified in the effluent and fish exposed to the undiluted effluent for 48-hours.

Compound	Effluent µg/l	Raw Fish* µg/kg
Chloroform	3	-
Carbon tetrachloride	32	1
Benzene	86	-
Toluene	50-100 ng/l	100
Xylenes	-	10
Styrene	-	6
Other	+	++

- not detected

+ high levels of petroleum hydrocarbons

++ alkanes (C_{14} - C_{26}) identified in both fish and effluent

* Composites of fillets from 4 fish

Table 8: Dow Chemical Third Street Sewer. Organic compounds identified in undiluted effluent and in raw and cooked fish (µg/kg) exposed to effluent for 48-hours.

Exposure Concentration	Effluent 100% (µg/l)	Raw Fish					Cooked Fish				
		Control	5%	10%	50%	100%	Control	5%	10%	50%	100%
Benzene	-	9.1	96.4	145	1,310	500	109	509	1,028	12,000	Sample Exhausted
Toluene	1.2	10	434	349	1,600	7,300	328	2,200	2,400	15,300	
Xylene & Ethylbenzene	6.3	9.4	206	482	1,700	6,100	198	611	1,154	17,000	
Styrene	*	-	347	905	8,600	17,000	-	1,200	2,300	41,400	
Diethylbenzene	10	-	27	136	660	9,000	-	80	2,300	32,000	
Napthalene	-	-	-	-	-	48	-	-	-	tr	
Methylnaphthalene	-	-	-	-	tr	15	-	-	-	tr	

- = non detected

tr = trace, 50 ng/kg 100 ng/kg

* = possibly lost by polymerization

NOTE: Each sample represents a composite of 4 fish fillets

Table 9: Ethyl Corporation Final Effluent. Organic compounds identified in raw and cooked fish (μg/kg) exposed to effluent for 48-hours.

	Raw Fish					Cooked Fish				
	Control	5%	10%	50%	100%	Control	5%	10%	50%	100%
Methylene chloride	-	-	Sample Exhausted	-	-	-	+	-	+	-
Dichloroethane	-	8.1		3,600	2,200	-	19.4	223	656	1,531
Toluene	12.8	193		165	44.3	-	17	71	108	109
Xylene & Ethylvenzene	tr	34.3		27	17.7	0.12	1.8	20	26.4	40
Cumene, Propylbenzene	-	21.4		28	-	-	+	+	29.2	28.4
Dichlorobenzene	-	-		4.4	-	-	-	-	-	-

tr = trace

\- = non detectable

\+ = compound present, not quantified

NOTE: Each sample represents a composite of 4 fish fillets

Table 10: Allied Chemical Final Effluent. Organic compounds identified and raw and cooked fish (μg/kg) exposed to effluent) for 48-hours.

	Effluent 100% (μg/l)	Raw Fish					Cooked Fish				
		Control	5%	10%	50%	100%	Control	5%	10%	50%	100%
Carbon tetrachloride	66	-	-	+	+	100	-	-	-	-	
Pentanol	-	570	870	650	530	-	210	260	280	230	
Toluene	-	-	tr	-	-	30	-	-	-	tr	
Hexanal	-	1,540	3,300	2,300	2,100	-	800	850	940	840	
Heptanal	-	280	440	350	330	-	140	150	170	170	
Pentylfuran	-	+	+	+	+	-	+	+	+	+	Sample Exhausted
Decanal	-	-	-	-	-	-	+	+	-	-	
Dichlorobenzene	-	-	-	490	600	-	-	tr	250	490	
Dichlorobenzene	26	-	570	1,130	3,200	4,300	-	66	570	4,150	
Trichlorobenzene	+	-	-	110	20	600	-	-	-	28	
Tetrachloroethylene	11	-	-	-	-	120	-	-	-	-	
Styrene	-	-	-	-	-	38	-	-	-	-	
Trimethylbenzene	-	-	-	-	-	19	-	-	-	-	
Naphthalene	-	-	-	-	-	9	-	-	-	-	
Methylnaphthalene	50	-	-	-	-	-	-	-	-	-	

tr = trace, 50 μg/kg to 100 μg/kg
\- = non detected
\+ = compound present, not quantified
NOTE: Each sample represents a composite of 4 fish fillets

the presence of organic compounds in fish and this is supported by the analytical evidence that volatile compounds identified in effluents were also identified in exposed fish. The lack of single compound tainting evaluations makes identification of tainting agents impossible in this study. However, threshold odour numbers for identified compounds in water have been used as a guide to associate tainting with specific compounds (Table 11).

The Esso Chemical Pressure Sewer with a tainting threshold of <5% would be expected to introduce into fish at least 12,500 µg/kg and 57,500 µg/kg of naphthalene and methylnaphthalene respectively. These values exceed the threshold odour numbers in Table 11 by factors of about 2000 which makes these compounds likely tainting candidates. Shipton et al. (1970) also indentified naphthalene and methynaphthalene in tainted fish. The panel described the fish as having an oily, hydrocarbon and gasoline flavour. The effluent had a similar strong odour.

The Imperial Oil Bio-oxidation Plant effluent again with a tainting threshold of <5% but unlike the previous case did not produce organic compound concentrations in fish that would account for tainting based on threshold odour numbers. The panel described the fish flavour as oily or petrochemical.

The Dow Chemical Third Street sewer also produced tainting in fish at 5% and at that concentration, levels of toluene (2,200 µg/kg) and styrene (1,200 µg/kg) were measured in cooked fish. These concentrations were factors of 100 and 2 respectively in excess of threshold odour numbers for those compounds. A styrene flavour to the fish was identified by the panel. Benzene toluene, xylene/ethylbenzene, styrene and dielthylbenzene were observed to increasingly accumulate in fish directly with exposure concentration, appeared to concentrate 1,000 to 10,000 fold compared to the effluent concentration and appeared in higher levels in cooked than raw fish samples. General comments by the taste panel indicated that fish exposed to this effluent were the most severely tainted of all those tested.

The Ethyl Corporation Final Effluent exhibited a 26% tainting threshold with only toluene (108 µg/kg) exceeding a documented threshold odour number in cooked fish exposed to 50% effluent. The panel, although capable of differentainting between 10% and 50% exposed fish, did not characterize the taste or register strong dislike for the fish flavour.

Table 11: Threshold odour numbers ($\mu g/l$) for selected compounds in water (7).

Compound	Threshold Odour Number	Comments
Benzene	31,300	detection at 40°C
1,2-Dichlorobenzene*	10	adverse fish taste 200 $\mu g/l$
Naphthalene	6.8	detection
1-methylnaphthalene	20	detection at 20°C
2-methylnaphthalene	10	detection at 20°C
Styrene	730	detection at 40°C
Toluene	17	compound recognition

* from Verschueren, 1977 (8)

The Allied Chemical Final Effluent also produced a 26% tainting threshold with none of the compounds appearing to contribute to tainting based on available information. Pentanol, hexanol, heptanal and pentylfuran were identified in relatively constant concentrations across the exposure gradient (including controls) and in raw and cooked fish which would suggest that they represent compounds not associated with the effluent. Dichlorobenzene is the only compound observed to accumulate in fish along the exposure gradient. The panel described the fish flavour as stale, rubbery, aromatic or similar to burnt almonds.

General

Several compounds appeared to play a major role in the toxicity and tainting of the exposed fish. Naphthalene and methylnaphthalene were the only compounds associated with both fish lethality and tainting while toluene and styrene appeared to contribute to tainting only. Benzene, xylene/ethylbenzene, diethylbenzene and dichlorobenzene although not associated with lethality or tainting in other studies demonstrated the capacity to bioconcentrate. The above compounds are probably not the sole causative agents of lethality and tainting but appear to contribute to the responses observed.

It was also observed that in several cases the concentration of organics in fish flesh increased upon cooking. The reason for this apparent concentrating effect is not evident although protein disassociation, release from lipids or general tissue decompartmentalization may play a role. The concentration of organics in fish is particularly important where fish are consumed through sport or commercial activities. Human health implications would arise when organic concentrations in fish approached response levels observed in other biological hazard studies (mutagenicity, reproductive impairment etc.,).

There is a need to determine the environmental fate of the above identified compounds. Similar studies are currently underway to determine the impact of solvent extractible compounds on fish. Since fish lethality is not a major problem with St. Clair discharges, investigations into sublethal effects of organics on aquatic biota should be continued (e.g. critical life stage evaluations, behaviour, preference/avoidance reactions).

The actual biological impact in the St. Clair River may be less than that predicted by application of this data in dispersion modelling since the experimental design of this study did not allow organisms to avoid perceptible stress.

CONCLUSIONS

1) Fish lethality was not a major feature of these effluents although most fish surviving the exposure showed signs of stress (darkening of the skin, loss of equilibrium, surface breathing).

2) Based upon 48-hour exposures of fish to the five industrial effluents tested there is a rapid and substantial uptake of organic chemicals. For example, styrene was not detected in the Dow Chemical effluent yet exposed fish accumulated 17,000 µg/kg of the compound in 48 hours.

3) Compounds most highly associated with fish lethality included naphthalene and methylnaphthalene while those associated with tainting included naphthalene, methlynaphthalene, toluene, and styrene. Benzene, xylene/ ethylbenzene, dielthylbenzene, dichlorobenzene, toluene and styrene demonstrated the ability to bioconcentrate in fish.

4) All effluents impaired fish flavour and three discharges tainted fish at effluent concentrations less than 5% v/v. However, the absence of taint in the fish does not necessarily represent a lack of organic chemical accumulation since some compounds may not be sensed by a taste panel.

5) Measured values of some contaminants were higher in cooked fish flesh than in raw flesh, although the reason for this is not known.

RECOMMENDATIONS

Future studies should attempt to determine:

1) The environmental fate of bioaccumulative organic compounds when relatively high levels of organics are identified in industrial discharges.

2) The human health implications of organics that bioconcentrate in sport or commercial fish should be investigated using other biological tests (e.g. mammalian and mutagenic studies).

3) Future aquatic toxicity studies should investigate sublethal responses of biota to organics (e.g. critical life stage evaluations, behaviour, preference/avoidance reactions).

BIBLIOGRAPHY

1. WELLS, D.L. 1972. Rubber manufacturing wastes and their effects on fish and water quality. Limnology and Toxicity Section, Water Resources Branch, Ontario Ministry of the Environment, Toronto, Ontario.
2. LAMBTON INDUSTRIAL SOCIETY WATER QUALITY SURVEY. 1976. Suite 201N, 242A Indian Road South, Sarnia, Ontario, N7Z 3W4. J.A. McCoubrey, Manager.
3. AMERICAN SOCIETY FOR TESTING AND MATERIALS. STANDARD PRACTICE TO EVALUATE AN EFFLUENT FOR FLAVOR IMPAIRMENT TO FISH FLESH. Draft copy available from:

   Mr. Howard C. Alexander
   Environmental Sciences Research
   Dow Chemical Company,
   1702 Building,
   Midland, Michigan 48640, U.S.A.
4. WILCOXON, F. and R.A. WILCOX. 1964. Some rapid approcimate statistical procedures. Published and distributed by Lederle Laboratories: A Division of American Cyanamid Company, Pearl River, New York.
5. MELANON, M.J. and J.L. LEECH. 1978. Distribution and elimination of naphthalene and 2-methylnaphthalene in rainbow trout during short and long term exposures. Arch. Environ. Contam. Tox. 7:207-220.
6. SHIPTON. J., J.H. LAST, K.E. MURRAY and G.L. VALE. 1970. Studies on a kerosene-like taint in mullet (Mugil cephalies) II: Chemical nature of the volatile constituents. J. Sci. Fd. Agric. 21: 433-436.
7. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1975. Aqueous odour thresholds of organic pollutants in industrial effluents. EPA-600/4-75-002.
8. K. VERSCHUEREN. 1977. Handbook of environmental data on organic chemicals. Van Nostrand Rienhold Co. Toronto.
9. GRAIG, G.R. and G.L. BEGGS. 1979. An evaluation of loading rates in regulatory static bioassays. Proceedings of the Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario. Eds. P.T.S. Wong, P.V. Hodson, A.J. Nimii, V. Cairns, V. Borgman.
10. BONNER, R.F. and Q. MERESZ. 1981. St. Clair River Organics Study. Identification and quantitation of organic compounds. Ontario Ministry of the Environment, Toronto, Ontario.